http://dx.doi.org/10.4314/ajtcam.v10i4.28

A STUDY OF EXTRACTION PROCESS AND IN VITRO ANTIOXIDANT ACTIVITY OF TOTAL PHENOLS FROM RHIZOMA IMPERATAE

Xian-rong Zhou*, Jian-hua Wang, Bo Jiang, Jin Shang, Chang-qiong Zhao

College of Life Science and Technology, Yangtze Normal University, Fuling 408100, P. R. China.
***E-mail:** zxrfsy@126.com

Abstract

The study investigated the extraction method of *Rhizoma Imperatae* and its antioxidant activity, and provided a basis for its rational development. The extraction method of *Rhizoma Imperatae* was determined using orthogonal design test and by total phenol content, its hydroxyl radical scavenging ability was measured by Fenton reaction, and potassium ferricyanide reduction method was used to determine its reducing power. The results showed that the optimum extraction process of *Rhizoma Imperatae* was a 50-fold volume of water, 30 ℃, three times of extraction with 2 h each. Its IC_{50} for scavenging of hydroxyl radicals was 0.0948 mg/mL, while IC_{50} of ascorbic acid was 0.1096 mg/mL; in the ferricyanide considerable reduction method, the extract exhibited reducing power comparable to that of the ascorbic acid. The study concluded that *Rhizoma Imperatae* extract contains relatively large amount of polyphenols, and has a good anti-oxidation ability.

Keywords: *Rhizoma Imperatae*, total phenol determination, hydroxyl radicals, potassium ferricyanide reduction method

Introduction

Originally recorded in the "Shen Nong's Herbal Classic", the *Rhizoma Imperatae* is the dried rhizome of the perennial herb *Imperata cylindrica*, which belongs to the Poaceae plant family. It is mainly grown in places such as Northern Africa, Turkey, Iraq, Central Asia, as well as China's Liaoning and Hebei. It is sweet in taste, cold in nature, and has the effects of cooling blood, arresting bleeding, clearing heat, and inducing diuresis. The main active ingredients of *Rhizoma Imperatae* include triterpenoids, coumarins, saccharides, organic acids, as well as iron, potassium, and calcium, which all have the haemostatic, diuretic, anti-bacterial, anti-inflammatory, anti-tumour and immune-regulatory effects. They are also commonly used in the clinical treatment of hematuria, glomerulonephritis, and jaundice. Recently, *Rhizoma Imperatae* has been widely applied in the development of healthcare products (Kaiser M et al., 2002; Lai Wah Chan et al., 2008; Yin You-sheng et al., 2011; Yue Xing-ru et al., 2006; Liu Rong-hua et al., 2010; Li Li-shun, 2011). In this paper, the water extract of *Rhizoma Imperatae* and its antioxidant activity were studied, thus laying a foundation for the study of *Rhizoma Imperatae* pharmaceutical and healthcare products.

Materials and Methods

Instruments

The main instruments include the following: UV-visible spectrophotometer UV-2100 (UNICO Instruments Co., Ltd.); digital thermostat water bath HH-8 (Guohua Electric Appliance Co., Ltd.); analytical balance FA1004 (Shanghai Jingke Balance).

Reagents

Rhizoma Imperatae was identified by Mr Giang Zhang of Central South University; the specimen was placed at the lab centre of the University.

Ferrous chloride, trichloroacetic acid (Tianjin Guangfu Fine Chemical Research Institute); ferrous sulphate (Tianjin Kemiou Chemical Reagent Co. Ltd., Batch number: 20080709); gallic acid (Ouhai Chemical Reagent Factory, Wenzhou Ouhai Fine Chemicals Corp); anhydrous sodium acetate (Tianjin Fengchuan Chemical Reagent Technology Co., Ltd., Batch number: 20091008); ascorbic acid (Tianjin Kaixin Chemical Industry Co., Ltd.); hydrogen peroxide (Heilongjiang Chemical Group Dahua Trade LLC. Batch number: 20110902); 1, 10-phenanthroline, pyrogallol (Tianjin Kemiou Chemical Reagent Co. Ltd.); potassium ferricyanide (Tianjin Damao Chemical Reagent Factory); anhydrous sodium carbonate (Tianjin Bodi Chemical Holding Co., Ltd., Batch number: 20111219); sodium phosphate (Sinopharm Chemical Reagent Co., Ltd.)

Determination of total phenols by Folin-Ciocalteu method (Pharmacopoeia of the People's Republic of China 2010)

Preparation of reference solution: 12.5 mg of gallic acid was weighed and placed in a 25 ml brown volumetric flask, then dissolved by

http://dx.doi.org/10.4314/ajtcam.v10i4.28

adding distilled water and diluted to the mark. 5 ml was precisely taken and placed in a 50 ml brown volumetric flask, and diluted to the mark with distilled water, shaken well, and 0.05 mg/ml gallic acid solution was obtained.

Drawing of standard curve

0.5 ml, 1 ml, 2 ml, 3 ml, 4 ml, and 5 ml of the reference solution were precisely measured and placed in 25 ml brown volumetric flasks respectively. The solution was added with 1 ml of Folin-Ciocalteu reagent plus 11.5 ml, 11 ml, 10 ml, 9 ml, 8 ml, 7 ml of distilled water respectively, and then diluted to the mark with 20% sodium carbonate solution, shaken well, and kept for 30 min. The absorbance was measured at 760 nm with the corresponding reagent as blank (that is, reference solution was replaced by sodium carbonate solution). The standard curve was drawn with reference solution concentration as the abscissa, and absorbance as the ordinate: $Y=0.1587X+0.2861(R^2=0.9985)$

Sample measurement

2 ml of *Imperata Cylindrica* extract was precisely measured and placed in a 25 ml volumetric flask, added with 1 ml of Folin-Ciocalteu reagent plus 10 ml of distilled water, and diluted to the mark with 20% sodium carbonate solution, shaken well, and kept for 30 minutes. The absorbance at 760 nm was measured three times with the corresponding reagent as blank (that is, reference solution was replaced by sodium carbonate solution) and averaged. Equivalent mg of gallic acid per gram of extract solution was then calculated from the standard curve.

Screening of optimum process for extraction of total phenols from *Rhizoma Imperatae* by orthogonal experimental design

Factors and levels: based on the results of single factor test in the pre-experiment, water was used as solvent, and ultrasonic extraction method was adopted. In the orthogonal design test, the amount of water (A), extraction time (B), extraction frequency (C), and extraction temperature (D) were selected as the study factors, and three levels were chosen for each factor. Optimum extraction conditions were determined with total phenolic content in *Rhizoma Imperatae* as the indicator. Factors and levels were shown in Table 1.

Table 1: Factors and levels of orthogonal test for *Rhizoma Imperatae*

Level	Factor			
	A Amount of water addition (fold)	B Extraction time(h)	C Extraction frequency (number of times)	D Extraction temperature (℃)
1	10	1	1	30
2	30	1.5	2	50
3	50	2	3	70

Anti-oxidation experiment

Determination of hydroxyl radical scavenging capacity by Fenton method (Liu Yang et al., 2012)

1 mL of 1,10-phenanthroline (2.5 mmol/L) was taken in the test tube, and sequentially added with 1 mL of PBS (150 mmol/L, pH 7.4) and 1 mL of distilled water. After mixing thoroughly, 1 mL of ferrous sulphate (2.5 mmol/L) was added, mixed well, then 1 mL of H_2O_2 (mass fraction of 20 mmol/L) was added. After water bathing at 37℃ for 1.5 h, its absorbance at 536 nm was measured and served as the absorbance of injury group Ap. 1 ml of H_2O_2 was replaced by 1 mL of distilled water and served as the absorbance of blank group Ab. Extracts with a series of concentrations of 0.1, 0.15, 0.2, 0.25, 0.3 mg/ml were taken, and 1 mL of distilled water was served as the absorbance of the sample group As. Absorbance value was measured using the same method and with ascorbic acid as the positive drug, and hydroxyl radical scavenging rate was calculated according to the following formula:

Hydroxyl radical scavenging rate = (As-Ap) / (Ab-Ap) × 100%

Its result was expressed in IC_{50}, that is, the concentration of the sample at 50% scavenging rate.

Potassium ferricyanide reduction method (Deng Chao-cheng, et al., 2010)

1 mL of different concentrations of sample (50, 100, 200, 400, 800 ug/mL) was taken and added to the test tube, and then added with 2.5 mL of PBS solution (pH 6.6) and 2.5 ml of 1% potassium ferricyanide solution. They were mixed well, water bathed at 50℃ for 20 min, added with 2.5 mL of 10% trichloroacetic acid solution, and allowed to stand for 10 min. 2.5 ml of supernatant was taken, added with the same volume of distilled water and 0.5 mL of 0.1% $FeCl_3$, and mixed well. After keeping at room temperature for 10 min, absorbance was measured at maximum absorption wavelength of 700 nm. The greater the absorbance value, the stronger the reducing ability.

Results

Total phenol content and orthogonal test results

Orthogonal test results

http://dx.doi.org/10.4314/ajtcam.v10i4.28

Table 2: Intuitive analysis results

Test No.	Amount of water addition A	Extraction time B	Extraction frequency C	Extraction temperature D	Total phenol content GAE(mg/g)
1	1	1	1	1	465
2	1	2	2	2	523
3	1	3	3	3	716
4	2	1	2	3	439
5	2	2	3	1	561
6	2	3	1	2	617
7	3	1	3	2	595
8	3	2	1	3	628
9	3	3	2	1	771
K1	568.000	499.667	570.000	599.000	
K2	539.000	570.667	577.667	578.333	
K3	664.667	701.333	624.000	594.333	
R	125.667	201.666	54.000	20.667	

It can be seen from the above table that the optimum extraction process was $A_3B_3C_3D_1$, that is, a 50-fold volume of water, 30 ℃, and three times of extraction with 2 hours each. Factors by degree of influence were B>A>C>D.

Analysis of variance and F-test results for the above table are shown in Table 3.

Table 3: Analysis of variance and F-test

Factor	Sum of squared deviation	Degree of freedom	F ratio	F critical value	Significance
Amount of Water addition A	25977.556	2	36.853	19.000	*
Extraction time B	62784.222	2	89.070	19.000	*
Extraction frequency C	5121.556	2	7.266	19.000	
Extraction temperature D	704.889	2	1.000	19.000	
Error	704.899	2			

"*" Indicates significant difference at a=0.05

The results show that the water amount and extraction time are statistically significant.

Determination of hydroxyl radical scavenging capacity by Fenton method

Table 4: Results for hydroxyl radical scavenging

	Concentration (mg/ml)	Inhibition rate (%)	IC_{50} (mg/ml)
Extract	0.1	20.15	0.0948
	0.15	35.67	
	0.2	50.92	
	0.25	68.97	
	0.3	91.16	
Vc	0.01	15.67	0.1096
	0.05	30.52	
	0.1	48.92	
	0.15	60.11	
	0.2	81.95	

It can be seen from the table that the IC_{50} of extract was 0.0948 mg/mL, while ascorbic acid had an IC_{50} of 0.1096 mg/mL.

Potassium ferricyanide reduction method

Table5: Results for potassium ferricyanide reduction method

Concentration (ug/mL)	Sample A_{700}	Ascorbic acid A_{700}
5	0.188	0.101
10	0.223	0.128
20	0.272	0.234
40	0.354	0.338
80	0.494	0.482

http://dx.doi.org/10.4314/ajtcam.v10i4.28

It can be seen from the table that absorbance values of the extract and ascorbic acid gradually increased with the increase of concentration, which was dose related. Reducing power of extract was gradually increased, which was slightly higher than that of ascorbic acid.

Discussion

Among numerous free radicals, ·OH is the most harmful to the human body. ·OH is the strongest known oxidant, and ·OH scavenging rate is an important indicator reflecting the antioxidant activity of drugs. Fenton system uses H_2O_2 and Fe^{2+} to produce ·OH, making aqueous solution of 1,10-phenanthroline-Fe^{2+} oxidised to 1,10-phenanthroline-Fe^{3+}, thus making the maximum absorption peak of 1,10-phenanthroline-Fe^{2+} at 536 nm disappear, and on these grounds identify the changes in the number of ·OH in the system. This method is simple and cheap, stable and reliable, but is not suitable for some sample extracts, such as samples which produce flocculent precipitates or interference colours during the experiments, as the accuracy of the experimental results will be affected (Fu Yu, 2010; Zhang Wen-bo, 2011).

Reducing ability is an important indicator of the antioxidant capacity of a substance, and one of the antioxidant mechanisms as well (Zheng Xiao-xian et al., 2009). Therefore, there is a certain correlation between the antioxidant activity and the reducing power. Samples with strong reducing power are good electron donors; their electron-donating ability can make Fe^{3+} reduce to Fe^{2+}, and make potassium ferricyanide $K_3Fe(CN)_6$ reduce to potassium ferrocyanide $K_4Fe(CN)_6$. Potassium ferrocyanide again reacts with Fe^{3+} to produce Prussian blue; the absorbance is measured at 700 nm wavelength in order to detect the production amount of Prussia blue as the reducing power of the sample. Samples having strong reducing ability can also participate in the free radical reaction, making the radicals become stable substances.

In this experiment, total phenolic content of *Rhizoma Imperatae* extract was determined by the Folin-Ciocalteu method and its antioxidant capacity by Fenton method and potassium ferricyanide reduction method. The results exhibited good antioxidant capacity in both of the systems. In today's green and healthy living era, application of natural antioxidants has gained increasing attention, and antioxidants extracted from plants (especially from Chinese medical herbs) have been more extensively used. Therefore, this experiment has laid certain foundation for future drug development.

Acknowledgements

This work was supported by the Natural Science Foundation Project of CQ CSTC (cstc2012jjA00006), the Fund of Chongqing Education Committee of China (KJ111318), and the ‘spring light project’ of the Ministry of Education of China (Z2011144)

References

1. Bijli KM, Singh BP, Sridhara S, Gaur SN, Arora N (2002). Standardizing Imperata cylindrical-source material for quality allergen preparations.Immunological Methods, 260:91-96.
2. Deng Chao-cheng, Huo Li-ni, Li Pei-yuan, Chen Rui, Deng Yan-hong, He Chun-ling, Lu Cheng-sheng (2010). Chemical Constituents and Antioxidant Activity of Essential Oils from Leaves of Cinnamomum burmannii in Guangxi.China Journal of Experimental Traditional Medical Formulae, 16 (17): 105-109.
3. Fu Yu (2010). Progress of Research on In Vitro Antioxidant Function Evaluation Methods,Meat Research, 11: 41-46.
4. Lai Wah Chan, Emily LC Cheah, Constance LL Saw, Wanyu Weng, Paul WS Heng (2008)Antimicrobial and antioxidant activities of Cortex Magnoliae Officinalis and some other medicinal plants commonly used in South-East Asia.Chinese Medicine, 3(1):15.
5. Li Li-shun, Shi Wei-jing, Wang Fu-cheng (2011) A Review of Chemical Constituents, Medicinal Function of Rhizoma Imperatae and Their Application in Health Care Products Development.Journal of Anhui Science and Technology University,25 (2): 61-64.
6. State Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China, Volume 1. China Medical Science Press, 2010. Appendix 62.
7. Liu Rong-hua, Fu Li-na, Chen Lan-ying, Ren Gang, Chen Shi-sheng, Chen Zhuo (2010).Chemical Constituents and Pharmacological Study Progress of Imperata cylindrical Rhizomes.Journal of Jiangxi University of Traditional Chinese Medicine, 22 (4): 80-83.
8. Liu Yang, Guo Xiao-yu, Sun Dian-kui, Tian Yi-xin (2012). Research on the antioxidant capability of the flavone extract from black soybean produced in different regions. Ginseng Research, 2012,1: 21-23
9. Yin You-sheng, Ou Jun, Wei Jia-zhi, Li Yun-qian, Li Xiao-li, Li Qing-chu, Li Kang-hui (2011) Effects of Rhizoma Imperatae and its Compound Decoction on the Model of IgA Nephropathy in Rats. Lishizhen Med. Mater. Med. Res, 22 (11): 2659-2662.
10. Yue Xing-ru, Hou Zong-xia,Liu Ping, Wang Shu-song (2006) Anti-inflammatory Effect of Imperata Cylindrica. Chinese Journal of Clinical Rehabilitation, 10 (43): 85-87.
11. Zhang Wen-bo. (2011)Research Progress on the Antioxidant Activity of Ingredients in Plant Samples in Vitro,Guangzhou Chemical Industry, 39 (24): 14-16.
12. Zheng Xiao-xian, Li Yan, Xu Wei-fei, Fang Yi-qun, Ran Yu-zhou (2009). Research on Antioxidant Activity of Yellow Rice Wine .Liquor-Making Science & Technology, 10: 57-64.